

Building and breaking the NPC

A study of nuclear pore complex (NPC) assembly is revealing Ran to be a ubiquitous regulator of nuclear physiology. RanGTP gradients are essential for nuclear trafficking, spindle assembly during mitosis, and reformation of the nuclear envelope (NE) after mitosis. Now, on page 1041, Ryan et al. implicate Ran in yet another nuclear function—NPC assembly.

In a screen for budding yeast NPC mutants, the authors identified several temperature-sensitive mutations in the Ran pathway, including mutations in a Ran guanine nucleotide exchange factor, a Ran GTPase activating protein, a nuclear importer of RanGDP, and the GTPase itself. Although preexisting NPCs were stable in the mutants, newly synthesized nucleoporin proteins (nups) were mislocalized at the restrictive temperature. Rather than localizing to the NE, several peripheral and integral membrane nups were found in vesicles that accumulated throughout the cytoplasm.

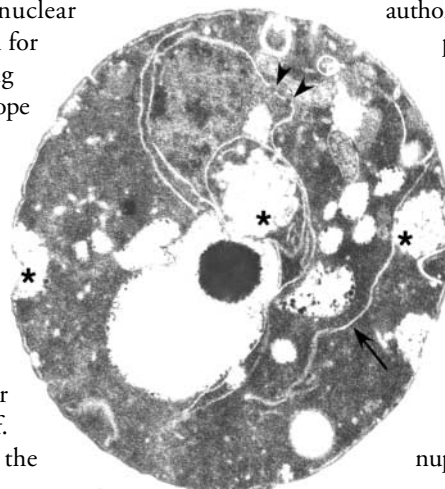
Thus, RanGTP may be involved in the fusion of vesicles at the nuclear envelope. This cytoplasmic Ran function contrasts with

the current dogma that GTP-bound Ran is primarily nuclear. The authors hypothesize that a yet unidentified GEF could produce RanGTP at the cytoplasmic side of the NE. Alternatively, the nuclear RanGEF Prp20 (mutations in which were isolated in this NPC screen) may have a small cytoplasmic pool that has so far gone undetected.

At the other end of the NPC lifecycle, a report on page 1055 by Lénárt et al. describes NPC disassembly as the initiating step in the breakdown of the NE preceding mitosis. The group identified two distinct phases of NE breakdown in starfish oocytes based on permeabilization of the nucleus.

During the first phase, the gradual release of nups from the NPC led to a small increase in NPC permeability. In the second phase, a wave of permeabilization spread rapidly from an epicenter-like origin, and gaps appeared in

the NE, possibly caused by removal of entire NPCs. Even at this point, the NE was still attached to the polymerized lamina along its inner membrane, indicating that disassembly of this network of intermediate filaments is a late step in NE breakdown. ■



In Ran mutants, nups get stuck in vesicles (*) rather than assembling into NPCs (arrowheads).

Calcium for strong clotting

Calcium fluctuations in the bottom layer of a platelet mass are propagated upwards to determine the size of a blood clot, or thrombus, according to results from Nesbitt et al. presented on page 1151.

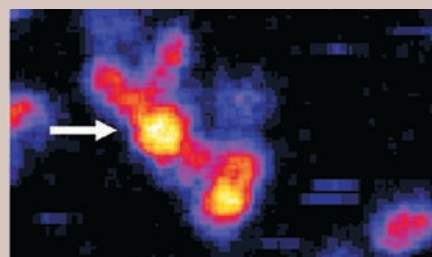
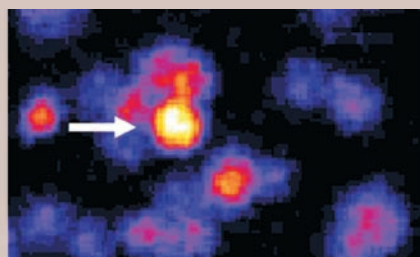
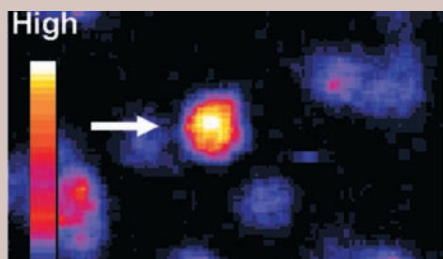
Platelets adhere to substrates in the vessel wall through receptors such as GPIb and certain integrins. Initial receptor engagement stimulates Ca^{2+} spikes in the platelets that lead to firm, permanent adhesion between the vessel wall and the primary layer of platelets. Now, Nesbitt et al. show that Ca^{2+} signals in this bottom layer are propagated among cells to promote platelet aggregation.

The group visualized cytosolic Ca^{2+}

concentrations in platelets and found that cells with strong Ca^{2+} oscillations efficiently nucleated platelet aggregates. Flowing platelets that contacted an anchored platelet at the peak of its Ca^{2+} fluctuation adhered and initiated their own Ca^{2+} oscillations. Propagation of the Ca^{2+} signals, which the authors call intercellular calcium communication, requires integrin $\alpha_{\text{IIb}}\beta_3$ and ADP, as well as the ADP receptor, P2Y_{12} . The group proposes that integrin activation tethers neighboring platelets, initiates Ca^{2+} spikes through release of stores in the newly adherent platelet, and stimulates local release of ADP. ADP activation of P2Y_{12} signaling in nearby

cells amplifies the Ca^{2+} flux in the nearby cells and sustains integrin-driven aggregation.

The results provide a model to explain how the vessel substrate collagen, which only contacts the first layer of adhering platelets, is nonetheless particularly effective at promoting thrombus growth. The group shows that compared with von Willebrand factor (vWf), another vessel substrate, collagen induced both stronger and more widespread Ca^{2+} oscillations in the primary layer of adhering platelets. As a result, collagen-based thrombi were over five times larger than those that formed on vWf. ■



Ca^{2+} oscillations passed among platelets promote clotting.